lar blood cell lineages (Muller-Sieburg and Sieburg, 2006). Could this reflect or determine their migration routes?

Pathogen products can recruit additional HSCs to tissues, but there must be antagonistic mechanisms and ways to stimulate their self renewal. Otherwise, chronic infections would exhaust our stem cell reserve. HSCs acting as scouts in peripheral tissues would not be expected to be constrained by molecules that inhibit their proliferation in the bone marrow. Moreover, there are age-related increases in HSC numbers and skewing of myeloid-to-lymphoid differentiation (Rossi et al., 2007). It will be interesting to determine whether migrating HSCs are particularly susceptible to aging. The seminal work of Massberg, von Andrian, and their colleagues is certain to catalyze further exploration of how migration of stem cells protects and replaces tissues.

REFERENCES

Adams, G.B., and Scadden, D.T. (2006). Nat. Immunol. *7*, 333–337.

Cheshier, S.H., Morrison, S.J., Liao, X., and Weissman, I.L. (1999). Proc. Natl. Acad. Sci. USA *96*, 3120–3125.

Kiel, M.J., Yilmaz, O.H., Iwashita, T., Yilmaz, O.H., Terhorst, C., and Morrison, S.J. (2005). Cell *121*, 1109–1121.

Massberg, S., Schaerli, P., Knezevic-Maramica, I., Kollnberger, M., Tubo, N., Moseman, E.A., Huff, I.V., Junt, T., Wagers, A.J., Mazo, I.B., and Von Andrian, U.H. (2007). Cell, this issue.

Muller-Sieburg, C.E., and Sieburg, H.B. (2006). Cell Cycle *5*, 394–398.

Nagai, Y., Garrett, K.P., Ohta, S., Bahrun, U., Kouro, T., Akira, S., Takatsu, K., and Kincade, P.W. (2006). Immunity *24*, 801–812.

Pappu, R., Schwab, S.R., Cornelissen, I., Pereira, J.P., Regard, J.B., Xu, Y., Camerer, E., Zheng, Y.W., Huang, Y., Cyster, J.G., and Coughlin, S.R. (2007). Science *316*, 295–298.

Rossi, D.J., Bryder, D., and Weissman, I.L. (2007). Exp. Gerontol. *42*, 385–390.

Winkler, I.G., and Levesque, J.P. (2006). Exp. Hematol. *34*, 996–1009.

Wright, D.E., Wagers, A.J., Gulati, A.P., Johnson, F.L., and Weissman, I.L. (2001). Science *294*, 1933–1936.

Wu, L., and Liu, Y.J. (2007). Immunity *26*, 741–750.

cis-Regulatory Elements within the Odorant Receptor Coding Region

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Complex regulatory mechanisms lead to the expression in each olfactory neuron of one allele of only one of the 1000 odorant receptor (OR) genes. In this issue, Nguyen et al. (2007) provide evidence that regulatory elements residing within the coding region of OR genes are involved in the singularity of OR gene expression.

Although there are over 1000 odorant receptor (OR) genes in the mouse genome, in any given olfactory sensory neuron, a functional protein is stably expressed from only one allele of one OR gene (Buck and Axel, 1991; Chess et al., 1994; Malnic et al., 1999). What is the mechanism that allows a single allele of a single odorant receptor gene to be expressed in each olfactory neuron? The large size of the OR gene family and the distribution of family members across most chromosomes make it difficult to fathom how the precision of this exclusivity is achieved. Findings by Nguyen et al. (2007) now suggest that elements within the coding region itself mediate exclusivity of OR gene expression.

Previous work has reported the exciting possibility that there is a single expression site for OR genes in the nucleus, which would help to explain the singularity of OR gene expression (Lomvardas et al., 2006). Chromosome capture and fluorescence in situ hybridization (FISH) experiments indicated that a conserved 2 kb region near an odorant receptor cluster on mouse chromosome 14, called the H region (Lane et al., 2002; Serizawa et al., 2003), was physically associated with the OR gene chosen for expression irrespective of its genomic location (Lomvardas et al., 2006). This led to the idea that the H region was a key regulator of the entire OR gene family.

Doubt was cast on this conclusion, however, by experiments knocking out the H region in mice and demonstrating that numerous scattered OR genes maintain seemingly normal expression (Fuss et al., 2007). In this knockout mouse, it was only the nearest OR genes that had their expression extinguished. Yet,

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these studies beg the question of whether the region of chromosome 14 remaining after H element removal is still physically interacting with the expressed OR gene. Continued interaction, despite the loss of the H element, would imply that there are additional sequences at this genomic location capable of associating with the expressed OR gene and involved in directing its expression. One must also consider other experiments in which extra H sequences were inserted elsewhere in the genome in transgenic mice, which allowed more than one gene to be expressed if one of them was a pseudogene (Lomvardas et al., 2006). Thus, the function of the genomic region that includes the H element might be accomplished by redundant sequences including H and nearby elements. Certainly, further analyses of this interesting region are warranted.

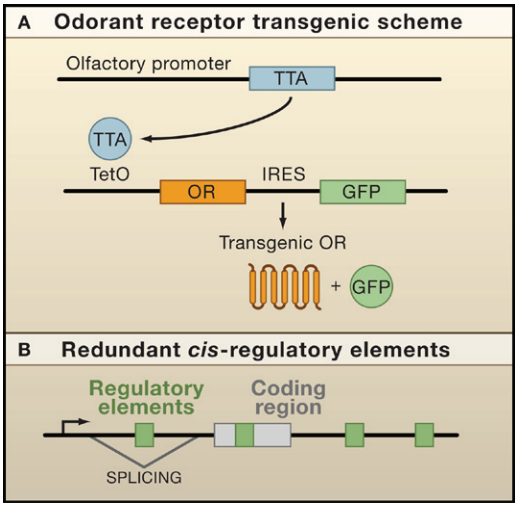

Figure 1. Regulation of Transgenic and Endogenous OR Gene Expression

(A) Transgenic mouse lines containing the tetracycline transactivator system. Expression of the Tet transactivator (TTA), driven by an olfactory neuron-specific promoter, activates the Tet-operator (TetO) *cis* element. This in turn drives expression of both an olfactory receptor (OR) gene and green fluorescent protein (GFP) via an internal ribosome entry site (IRES).

(B) In a generic odorant receptor gene, regulatory elements (green boxes) are dispersed throughout the gene including one in the coding region (gray) along with several others found elsewhere. Alternative models suggest that these regulatory elements are either involved in the suppression of nonexpressed OR genes or are involved in OR gene activation.

In this issue, Nguyen et al. (2007) take a different approach to probing the mechanisms that olfactory neurons use to regulate OR gene expression. Other investigators have focused on manipulating endogenous OR genes in the context of their normal regulatory regions or have made transgenic constructs containing endogenous genes and flanking DNA. In contrast, Nguyen et al. use an artificial system to exogenously drive expression of OR coding regions (Figure 1).

In this experimental scheme, mice express transgenic OR genes under the control of the Tet transactivator (TTA) system. The TTA system was used because initial efforts in which olfactory neuron-specific promoters were placed immediately upstream of OR coding regions failed to express the transgene. This suggested that proximity of the OR coding region was downregulating olfactory neuron-specific promoters. So the key element of the TTA system is that it physically separates the olfactory promoter (Gγ8 or OMP) from the transgenic OR coding region (Figure 1).

The Gγ8 promoter is expressed early in the maturation of olfactory neurons before the choice of an OR gene has been made, whereas the promoter for the olfactory marker protein (OMP) is expressed late in olfactory neuron maturation, after the OR gene has been chosen. Gγ8-TTA and OMP-TTA transgenic mice were crossed with mice carrying transgenes in which either the M72 or the rI7 OR gene is placed downstream of the Tet-operator (TetO) *cis* element that responds to TTA to drive expression of the OR gene. Thus, the transgenic OR coding region is physically separated from the transgenic olfactory promoter. Expression of the OR transgene was then tracked by expression of a marker, either green fluorescent protein (GFP) or lacZ placed within the TetO construct (Figure 1).

Nguyen and colleagues find that transgenic rI7 and M72 OR genes under the control of the Gγ8 promoter, which drives transcription early in neuronal maturation, are chosen for expression by a high fraction of olfactory neurons. Within these neurons that are expressing the transgenic OR gene, the endogenous genes appear to be silent. However, when under the control of the late-expressing OMP promoter, the transgenic OR genes are chosen less frequently. In these 10%–30% of neurons that express the transgenic OR gene, endogenous genes are not expressed, indicating that expression is mutually exclusive. By creating a transgenic mouse in which the OR gene is expressed under the control of both the Gγ8-TTA and the OMP-TTA promoters, the authors were able to control initial choice and stable expression of the transgenic OR in up to 90% of olfactory neurons. Gγ8-driven precocious expression of TTA ensures that the olfactory sensory neuron choice is monopolized by their transgene, and that this expression is maintained by the later-activating expression of TTA by OMP. These experiments indicate that transgenic OR genes, in some respects, fall under the control of the cell's endogenous mechanisms for OR gene choice, despite the fact that the TTA system is unrelated to mammalian regulatory systems.

Published studies—for example by Serizawa et al. (2003) and Shykind et al. (2004)—can be considered in the context of competition for a singular site that allows expression. However, the TTA-based transgenic approach of Nguyen et al. is sufficiently distinct from endogenous regulatory elements that it is reasonable to consider it as driving expression independent of the regular OR gene choice machinery. Now consider the two main observations reported by Nguyen et al. (2007): first, exogenous transgene-driven OR gene expression can exclude expression of the endogenous OR gene repertoire; and second, the transgene can

itself be excluded by prior expression of an endogenous OR gene. The first observation is consistent with the idea raised by earlier transgenic experiments that the expression of a functional OR gene can exclude expression of other OR genes. The second observation leads to the conclusion that the OR coding region contains important regulatory sequences, which could take one of two exclusive forms. Such elements could be involved in the suppression of nonexpressed OR genes. By an alternative model, discussed below, the potential function of these elements would be to allow interaction of a given OR gene with a singular activating expression site. Why then did earlier transgenic and knock-in experiments that removed the coding region not lead to increased expression or, by the alternative model, lead to ablated expression? The answer could be redundancy. Perhaps these *cis* elements are distributed such that inclusion of an entire OR coding region is likely to bring along with it one or more such elements, whereas deletion of the coding region of a larger transgenic construct (or at an endogenous locus) would not remove all such elements (see proposed model in Figure 1B).

Of course, one also has to consider the possibility that the transactivator system, when driving an OR gene, could in fact rely upon aspects of the endogenous mechanism for choosing an OR gene. This would dictate that when the endogenous mechanism has already chosen an endogenous OR gene prior to TTA production, the TetO promoter is somehow inhibited from its normal stimulation by TTA. This would suggest the interesting possibility that the OR coding sequence contains at least one *cis*-regulatory element that allows association with the endogenous OR gene choice mechanism. Other sequences must also allow for similar associations because of the ability of OR genes that lack coding regions to be chosen by the endogenous OR gene choice mechanism.

Nguyen et al. also present results to address whether an olfactory neuron has the capacity to express multiple ORs. By using an internal ribosome entry site (IRES) to create a transgene that expresses two ORs in tandem, they were able to induce coexpression of two ORs and showed that they could both mediate physiologic responses. Thus, the exclusive expression of one OR is not due to an inability of the cell to support multiple functional ORs but rather is due to layers of gene regulation.

Recent work on OR gene regulation has certainly raised interesting issues for further experimentation. We have suggested explanations that attempt to bring together the various published results, although there are other possible explanations. Future work will clarify the mechanisms underlying OR gene choice. These fascinating mechanisms may also shed light on gene regulation in other systems.

REFERENCES

Buck, L., and Axel, R. (1991). Cell *65*, 175–187.

Chess, A., Simon, I., Cedar, H., and Axel, R. (1994). Cell *78*, 823–834.

Fuss, S.H., Omura, M., and Mombaerts, P. (2007). Cell *130*, 373–384.

Lane, R.P., Roach, J.C., Lee, I.Y., Boysen, C., Smit, A., Trask, B.J., and Hood, L. (2002). Genome Res. *12*, 81–87.

Lomvardas, S., Barnea, G., Pisapia, D.J., Mendelsohn, M., Kirkland, J., and Axel, R. (2006). Cell *126*, 403–413.

Malnic, B., Hirono, J., Sato, T., and Buck, L.B. (1999). Cell *96*, 713–723.

Nguyen, M.Q., Zhou, Z., Marks, C.A., Ryba, N.J.P., and Belluscio, L. (2007). Cell, this issue.

Serizawa, S., Miyamichi, K., Nakatani, H., Suzuki, M., Saito, M., Yoshihara, Y., and Sakano, H. (2003). Science *302*, 2088–2094.

Shykind, B.M., Rohani, S.C., O'Donnell, S., Nemes, A., Mendelsohn, M., Sun, Y., Axel, R., and Barnea, G. (2004). Cell *117*, 801–815.